EARLY DETECTION OF HALLMARK MOLECULAR DEPOSITS IN THE RETINA FOR DEMENTIA AND RELATED NEURODEGENERATIVE DISORDERS

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Epidemiological data

Dementia is an untreatable chronic and progressive syndrome affecting cognition, memory and motor skills in elderly and leads to incapacity of self-sustenance and death in late stages \([1,2]\). Currently, the World Health Organisation (WHO) estimates that 47 million patients suffer of dementia and almost 10 million new cases are diagnosed annually. Ageing is considered to be the main non-modifiable risk factor for dementia and with constantly increasing life expectancy and ageing population, WHO predicts 135 million patients by 2050. Consequently, in 2017 WHO initiated the ‘Global action plan on the public health response to dementia 2017-2025’ for providing early diagnostic methods, increasing public awareness, and promoting treatment initiatives and new research developments in the field \([2]\).

Brief overview on hallmark proteins

Dementia collectively denominates several forms of neurodegenerative diseases which clinically evolve to similar symptoms. Alzheimer’s disease is estimated to be responsible for up to 70% of dementias, but vascular forms or dementia characterised by fronto-temporal brain degeneration and dementia characterised by the presence of Lewy bodies are also documented \([2]\). Early diagnosis plays crucial role in preventing development of clinical symptoms and opens new prospective for early phase clinical trials, in contrast to prevailing practice in the present.

Vascular dementia also termed vascular cognitive impairment has a vascular aetiology that leads to neuronal loss. Early diagnosis of vascular abnormalities is possible through non-invasive imaging. Vascular imaging underwent a quick development in the past decade partially because of already available technology, the greater size of imaged structure (the blood vessels) and the easy access to vascularised neuronal tissues. Avoiding contrast agents for angiography of the brain, the retinal imaging became a non-invasive, vastly available, cost-effective and patient-friendly procedure. It is also capable of imaging fine structures such as focusing on arterioles, venules and capillaries. Vascular abnormalities and morphological changes in the retina have been well characterised and previously summarised \([3,4]\). In this review, we do not intend to repeat previous works, but rather focus on the latest advancement in non-invasive imaging of molecular biomarkers such as retinal deposits, hallmarks for neurodegenerative disorders leading to cognitive impairment. However, it is important to mention that vascular and structural changes in the retina were found to be associated with hallmark protein burden in elderly patients \([5]\).

Alzheimer’s disease received the most focus because of the high prevalence among patients with cognitive impairment. Consequently, research in Alzheimer’s disease brought major advancements in understanding the basis of this form of dementia. Non-modifiable risk-factors such as genetic heritage or ageing and modifiable risk-factors including life style, cardiovascular condition, metabolic status and depression were recognised, and preventive actions became possible \([1]\). Besides recognising risk factors, investigating the aetiology of dementia improves our capacity to develop diagnostic and treatment tools. Researching the molecular basis of Alzheimer’s disease, several hallmark proteins were discovered in the brain. Amyloid-beta (AB) and its pathological enrichment form extracellular senile plaques \([6]\) and hyperphosphorylated tau aggregates in neurofibrillary tangles \([7]\). These protein enrichments were found to be responsible for cellular signalling malfunction, neuronal loss and lead to development of clinical symptoms including cognitive decline \([8,9]\). These proteins are not restrictive to Alzheimer’s disease, and tau lesions proved to be involved also in fronto-temporal dementia \([10]\). Step by step, different classes of protein aggregates were identified in the brain in patients suffering of cognitive decline. Lewy bodies were identified to be
the hallmark cytoplasmic inclusions for a separate category of dementia which later became known as dementia with Lewy body. Here, the major protein constituent of the aggregate is α-synuclein [11,12]. Moreover, Spillantini and colleagues reported that pathologic α-synuclein deposits were present in both dementia and another neurodegenerative disease with cognitive decline, Parkinson’s disease; however, the α-synuclein inclusions were localised in separate specific brain regions. The burdens of these neurodegenerative diseases are overlapped, and the aetiology causing specific onset order for cognitive or motor symptoms remains subject of controversies [12,13]. Further research on α-synuclein revealed that the protein also forms inclusions in multiple system atrophy (MSA), which is another neurodegenerative disease with neurological symptoms including cognitive decline. Histologically, MSA can be characterised by the Papp-Lantos body which is a pathological build-up of α-synuclein and several other molecules in olygodendroglia cells [14-16]. The pathomechanism is not fully understood, but available research data shows that α-synuclein and tubulin polymerization promoting protein (TPPP/p25), an interacting olygodendrocyte specific protein, are major players in pathological deposit formation [16,17]. Firstly, the physiological function of TPPP/p25 in olygodendrocytes was identified and the protein was termed accordingly [18]. Later, observations were made on its function and involvement in diseases and TPPP/p25 was found to aggregate in Lewy bodies of Parkinson’s disease, diffuse Lewy body diseases and multiple system atrophy, and interacts with α-synuclein [17,19].

The pathological accumulation of hallmark proteins and disruption of physiological processes in the central nervous system is a slow and long-lasting mechanism. It was found that Aβ deposits may be present in the brain 15 to 20 years prior to development of clinical symptoms and are the first detectable signs for dementia [20,21]. In this sense, it is reasonable to develop diagnostic tools targeting pathological brain deposits of hallmark proteins. The above-mentioned proteins are not extensive to all major hallmark deposits in the brain in neurodegenerative diseases and many more brain proteins and molecules were found to be involved in neurodegenerative disorders with cognitive decline. However, the mentioned ones are currently subject to the latest advancement in retina research.

**Hallmark deposits in the retina**

Amyloid-beta plaques were identified in mouse models of Alzheimer’s disease and diseased human retinas by immunohistochemical procedures in post-mortem tissue samples [22,23]. Furthermore, studies have shown that amyloid-beta plaques in retina correlate with Aβ brain deposits in these patients [22,24] and their presence in the retina has a 100% sensitivity for Alzheimer’s disease [23]. The previous immunohistochemical research was conducted on cadaveric retina and brain tissue samples. Phosphorylated tau protein was also found in transgenic mice models of Alzheimer’s disease and diseased human retinas processed for immunohistochemistry [25]. In the last decade, independent studies brought evidence to support the possibility of imaging the retina for hallmark proteins such as amyloid-beta or phosphorylated tau for early diagnosis of neurodegenerative pathologies. Furthermore, studies reported the expression of several other brain specific proteins with possible pathological function in the retina which are currently under investigation. α-synuclein has been found to form Lewy bodies in the inner retina of patients suffering of Parkinson’s disease, and extensive immunoreactivity was also detected in the inner plexiform layer [26]. In comparison, the study reports no α-synuclein aggregates in samples from healthy controls. TPPP/p25 protein was first identified in the retina a decade ago [27]. However, investigation on its specific sub-cellular localisation and its possible role in the inner-retina were recently reported [28]. Future studies may identify the role of α-synuclein and TPPP/p25 in neurodegenerative conditions of the eye. As previously demonstrated for amyloid-beta, α-synuclein and TPPP/p25 burden in the retina and brain could be investigated for possible correlation. If this is the case, the two proteins may become new candidates for non-invasive imaging hallmark proteins in neurodegenerative conditions.

Similarly to the brain, pathological deposits in the retina may aggregate multiple proteins and micro-elements in plaques. An example of such is drusen, a pathological deposit that occurs at the interface of Bruch’s membrane and the pigment epithelium cell layer of the retina and its presence is age dependent. These deposits are the hallmark for age related macular degeneration (AMD), a neurodegenerative disease of the retina which is responsible for 8.7% of blindness worldwide and the most cases of vision loss in the UK [29,30]. The main reason for this, is the insufficient treatment due to unknown aetiology of the disease. However, recent investigation identified hydroxyapatite spherules to form the core of drusen deposits in the ageing eye which may further trigger the aggregation of proteins on the surface of spherules resulting plaques [31]. Ohno-Matsui summarised parallel findings in AMD and Alzheimer’s disease, including the presence of Aβ in plaques in cases of both age related neurodegenerative diseases [32]. Furthermore, a separate study reported diminished cognitive function in patients presenting drusen deposits in the retina [33], raising the possibility of very early detection of signs that lead to cognitive dysfunction. Based on these studies, earliest detection of drusen formation may be beneficial for both AMD and cognitive impairment.

**Imaging the retina - Latest advancements**

The retina proved to be an excellent target tissue for searching for the earliest changes in dementia. It is suitable for detection of finest vascular changes at capillary level and morphological changes that have been
shown to correlate with Alzheimer’s disease and several cognitive neurodegenerative disorders with cognitive decline [4,34,35]. However, when it comes to detecting molecular aggregates, the challenge exceeded the difficulty of imaging the blood vessels of the retina with existing, and widely available instruments (ophthalmoscope, biomicroscope or ocular coherence tomography) until recently.

Koronyo and his colleagues reported in 2017 a breakthrough in imaging Aβ deposits in human retinas of living patients suffering of Alzheimer’s disease. These patients were required to ingest a non-toxic oral supplement of curcumin for several days, followed by non-invasive imaging of the retina with a modified ophthalmoscope and optical coherence tomography [36]. Curcumin labelled amyloid plaques were visible across the retinas of patients suffering of Alzheimer’s disease in contrast to healthy controls. This technique was previously tested in rodent models of Alzheimer’s disease. After in vivo imaging, the animals were sacrificed and immunohistochemical labelling confirmed the presence of amyloid-beta plaques [22,37]. Curcumin is known to bind amyloid plaques and neurofibrillary tangles [36,38]. This technique seems very promising in early detection of hallmark proteins for Alzheimer’s disease such as amyloid-beta. However, reported limitations are represented by lipofuscin aggregates responsible for auto-fluorescent spots in elderly eye and few other causes of auto-fluorescence may hide the curcumin signal [36,39]. This may cause difficulties in discriminating curcumin labelled Aβ plaques and consequently leads to wrong diagnosis.

A separate study group reported lack of success in detecting curcumin labelled Aβ deposits in living patients of early onset Alzheimer’s disease [40]. The researchers used a multispectral scanning laser ophthalmoscope for imaging retinas of patients after 5 days of oral curcumin supplementation. Here, the daily dosage was 180mg curcumin, versus 1g daily intake in Koronyo’s study. The lack of labelling was speculated to be caused by low curcumin uptake or high auto-fluorescence compared to curcumin labelling, absence of Aβ deposits in the studied patients or plaques in retinal region off the scanned areas [40]. Whichever the reason, it is clear that this non-invasive imaging technique of hallmark proteins for neurodegeneration is a very promising direction for early diagnosis. However, optimisation for reproducibility should be done.

The detection of drusen in the central or peripheral part of the retina is an easy task for ophthalmologists worldwide. Ultra-wide field imaging techniques make detection and follow-up possible across whole retina. Although drusen deposits are visible during retinal examination through an ophthalmoscope, retina-photography or ocular coherence tomography, the earliest stages of hydroxyapatite spherule formation are unnoticed. A breakthrough in the field however is on the horizon through the advancements brought by Dr R. Thompson, Dr I. Lengyel and colleagues. The researchers reported the possibility of imaging hydroxyapatite deposits formation in the human retina. Once again, the labelling agent is a non-toxic, well characterised agent used for decades in medicine, tetracycline [41]. This compound is well known for its bounding capacity to hydroxyapatite. The researchers investigated whether the tetracycline has the capacity to label hydroxyapatite in drusen deposits in cadaveric retinas, and if signal can be detected by fluorescence lifetime imaging microscopy (FLIM). Their experiment concluded that tetracycline is suitable to stain hydroxyapatite spherules in the retina and the imaging technique captures the signal. This observation is of high value, combined with a previous report where fluorescent lifetime measurements were performed in living human retinas using fluorescent lifetime imaging ophthalmoscopy [42].

Is the retina our best option?

Not a single evident diagnostic tool is available for identifying the type of dementia by aetiology and the set of tests applied have different sensitivities and specificities depending on stage of the disease. Doctors usually indicate a set of tests, clinical and paraclinical examinations to diagnose and evaluate the stage of dementia, which usually are expensive, not suitable for screening in early stage and may be unavailable in several geographic regions.

A set of tests are available for screening the mental status of patients and these are widely used, even in stages when patients are not aware of cognitive decline yet. Mini Mental State Examination (MMSE), the Mini-Cog and the MoCA tests are adequate for diagnosing cognitive impairment and dementia, and several others were extensively presented by Bart Sheehan [43].

However, assessment of biomarkers is needed for recognising the aetiology and type of dementia, in order to initiate available treatment or recommend further lifestyle changes to reduce disease progression. It would be ideal to identify biomarkers present in the central nervous system 15-20 years prior to symptomatic stage.

Among the most advanced methods for imaging biomarkers already in clinical practice is the Positron Emission Tomography (PET) for amyloid and tau imaging in the brain of dementia patients. Using this technique, pathological deposits of amyloid-beta or tau can be detected in diseased or clinically healthy individuals, presumably in the pre-symptomatic phase of dementia [21,44]. Further, a variety of imaging or biochemical biomarkers are available, and an extensive overview is presented in Sen’s paper [44]. Acknowledging the importance of these techniques, there are several disadvantages in their use: not widely accessible, expensive and are time consuming examinations. There are also risk factors when using some of the techniques, such as radioactivity of the tracers in PET scanning and invasive aspects of several techniques.
(venous or lumbar puncture) may trigger infections, local inflammation and haemorrhages to mention a few.

In contrast to these, imaging the retina ensures a non-invasive technique to directly visualise a neuronal tissue and detect hallmark deposits that may develop 2 decades before cognitive decline and other clinical symptoms. Imaging amyloid-beta deposits in the retina could be performed by technicians, optometrists and ophthalmologists, in contrast to detecting biomarkers from cerebrospinal fluid where the lumbar puncture would be performed exclusively by experienced physicians. The fluorochrom agents used for imaging the retina (curcumin and tetracycline so far) are safe compared to the radioactive tracers used during PET scans. Also, the resolution of the acquired pictures is significantly greater in the retina compared to PET scans of amyloid and tau in the brain. Moreover, incorporated OCT (as described in Koronyo’s study) provides high resolution images of the neurosensory retina and its vasculature, making possible the detection of morphological changes in the vicinity of plaques. Developed countries run screening programmes (diabetic retinopathy screening for example) and automated data analysis of images by software are tested for further reducing examiner-related costs. Adding the hallmark deposit imaging of neurodegenerative disorders to existing eye-screening programs, may further strengthen management of elderly healthcare.

**CONCLUSION**

As routine or blood tests are ordered for screening, diagnostic or follow-up purposes, imaging the retina is expected to become the screening, diagnostic and follow-up tool for several neurodegenerative disorders in the near future. Cutting edge technology pushes the boundaries of cellular and sub-cellular level imaging of retinal neurones in clinically healthy or diseased patients, conferring new hopes both for early diagnosis and delaying disease progression. These new imaging methods for detecting hallmark molecular deposits in the retina for dementia and related neurodegenerative disorders are expected to be non-invasive, patient-friendly, quick, repeatable and cost-effective in the management of elderly healthcare.

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